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The orphan nuclear receptor Nr2e3 plays dual functions in rod photoreceptor differentiation

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During retinal development, rod and cone photoreceptors are generated from common pools of neuroepithelial progenitors. Nrl, Crx, and Nr2e3 are the key transcriptional regulators that control rod differentiation. Nr2e3 is a rod photoreceptor specific orphan nuclear receptor. However, the loss of its function results in enhanced S-cones and rod degeneration in both human (ESCS patients) and mice (rd7) retina. Nr2e3 is not detected in the Nrl−/− retina, which exhibits excess functional S-cones at the expense of rods. Here, we show that, using GFP to tag the newborn rod precursors, some early born rod precursors are transformed into S-opsin-positive cells in the rd7 mouse. On the other hand, forced over-expression of functional Nr2e3 in postmitotic cone precursors induces them to adopt rod pathway at the expense of cone differentiation. However, these new rod-like photoreceptors are not functional, partially due to the lack of rod transducin. The dual functions of Nr2e3 on rod and cone gene regulation are not dependent on Nrl and/or Crx but rely on its expression time and level. Thus, our in vivo studies reveal a critical role of Nr2e3 in rod photoreceptor differentiation during retinal development.

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Misexpression of neuroD in the developing zebrafish retina: Effect on proliferation and photoreceptor genesis

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NeuroD is a member of a large family of proneural genes and has been implicated in retinal cell genesis, neuronal development, and cell cycle regulation. In the retinas of larval and adult teleosts, neuroD is expressed in two post-mitotic cell populations, a subset of amacrine cells and transiently in nascent cone photoreceptors, and proliferating cells of the rod and cone photoreceptor lineages. In contrast to other vertebrate retinas, neuroD is not expressed in multipotent progenitors, indicating that in teleosts neuroD is not determinative for retinal cell fates. This makes the zebrafish retina a unique system to study the role of neuroD in lineages of cells, which give rise exclusively to photoreceptors. To test the function of neuroD in these cells, lines of zebrafish transgenic for heat shock 70/4:neuroD-EGFP (Hsp:nrd-EGFP) were established for conditional gain-of-function experiments. Heat shock resulted in robust EGFP fluorescence (37°C, 60min) throughout the embryo. Embryos were evaluated by in situ hybridization with probes to neuroD and islet1. Cell type-specific antibodies were used to label amacrine cells, cone, and rod photoreceptors, respectively. Mitotically active cells were labeled with 5mM BrdU and 15% DMSO. Heat shock induces expression of message for neuroD and islet1, a downstream target of neuroD, and results in marked decrease in proliferation compared to controls. We conclude that the Hsp:neuroD-EGFP construct generates functional protein in vivo, and conditional misexpression suggests that neuroD may modulate the mitotic activity of cells in rod and cone photoreceptor lineages.

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Photoreceptor subtype specification and mosaic patterning in zebrafish

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The well characterized, laminar organization of the seven major cell types of the vertebrate retina has been indispensable for investigating neural development. Unfortunately, the genetic mechanisms underlying the true diversity of neuronal subtypes and their non-random or mosaic arrangements within each lamina remain poorly understood. We have taken advantage of the precisely defined photoreceptor mosaic of the teleost to investigate cell–cell signaling mechanisms essential for subtype specification and patterning in the retina. In zebrafish embryos mutant for the neurogenic gene *mindbomb* (*mib*), cells in the outer nuclear layer adopted a default, red cone fate. Genetic chimera analysis showed that mib mutant cells retained the potential to differentiate into the full repertoire of photoreceptor subtypes consistent with a model of lateral inhibition. However, following genetic perturbations of neurogenesis, the photoreceptor mosaic pattern was quickly re-established in regions of newly differentiated neurons demonstrating that appropriate spacing is not dependent upon propagation of a wave of signaling from a pre-existing photoreceptor cell mosaic. Furthermore, pharmacological inhibition of Notch signaling produced more modest disruptions of retinal organization consistent with the absence of Müller cell maturation. Unexpectedly, the putative radial glial cells of the inner nuclear layer did not differentiate but reentering the cell cycle. Our results demonstrate new context-dependent roles for notch signaling in cone photoreceptor subtype specification, and we are continuing our screen for mutations affecting photoreceptor subtype specification.

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Role of senseless in late color photoreceptor differentiation in Drosophila

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Color vision requires the comparison of the output of classes of photoreceptor cells (PRs) that respond differently to wavelengths of incoming light. In *Drosophila*, color vision is achieved by the two inner PRs: R7 and R8, which express different rhodopsins (Rhs). The purpose of our studies is to investigate the role of senseless (sens) in late color photoreceptor differentiation. *Drosophila* sens (vertebrate Gfi-1) encodes a zinc finger transcription factor that is necessary for R8 specification in early eye development. From our expression studies and misexpression experiments, Sens also appears to induce R8-specific characteristics and repress R7 characteristics during late photoreceptor differentiation. To clarify the role of Sens in distinguishing R8 from R7 cells, we are addressing how Sens regulates the transcription of rhodopsins using luciferase assays and gel shift analyses. Moreover, we are testing the expression of rhodopsins in photoreceptor lacking Sens late in eye development. The studies will provide insights into the role of Sens in regulating rhodopsin expression and inner (color) photoreceptor differentiation in the adult *Drosophila* eye.

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Mapping the functional domains of Otd during eye development in *Drosophila*

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The *Drosophila* homeobox transcription factor Orthodenticle (Otd) is critical for eye development. Like the mammalian homolog Cone-rod homeobox (Crx), Otd is expressed in photoreceptor cells and is essential for correct photoreceptor cell morphogenesis and opsin gene regulation. Interestingly, Otd can function as both an activator and repressor of rhodopsin genes in a cell specific manner. The purpose of this study is to understand more about how the Otd protein regulates gene expression by mapping its various transcriptional regulatory domains. To test which parts of the protein participate in activation or repression of rhodopsins, we are testing Otd deletion constructs with both in vitro and in vivo approaches. In vitro luciferase experiments will analyze activation of the blue-sensitive rhodopsin 5 encoding gene to look for domains that participate in activation. To confirm these domains in vivo, we use the Gal4-UAS system to evaluate the ability of these deletion constructs to regulate rhodopsin 5 in otduvi mutant transgenic flies. The in vivo analysis will also reveal domains that participate in the repression function of Otd measured by repression of green-sensitive rhodopsin 6 encoding gene. Currently, in vitro experiments have identified two domains that participate in rhodopsin 5 activation. These results were confirmed with in vivo experiments. Ongoing studies are investigating repression domains.

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Role of Pros/Prox1 during lens development in *Drosophila*

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The homeodomain protein Prospero is expressed in the presumptive R7 photoreceptor and the lens-secreting cone cells during retinal development in *Drosophila*. At some point during pupal development, localization of Pros in cone cells switches from nuclear to cytoplasmic. We wish to understand when this switch occurs, how it is mediated, and whether it has any relation to terminal events of cone cell differentiation. We also wish to determine if early nuclear expression in cone cells plays a role in their specification and/or survival. Initial experiments have revealed that Prospero does play a role in cone cell development and that early expression patterns are dynamic in various cell types within the developing *Drosophila* retina. The successful completion of this work will lead to a better understanding of the role of Prospero in cone cell development. Interestingly, the vertebrate homolog of Prospero, Prox1, has also been associated with lens development. Therefore, this work may also provide insight into the mechanisms underlying lens formation in the vertebrate eye.

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Lens: A ground state for all sensory placodes?

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Cranial placodes are focal thickenings in the head ectoderm that contribute to cranial sensory ganglia and the paired sense organs. All placodes generate neurons, except for the lens. At neurula stages, precursors of all placodes are found in a continuous domain of the ectoderm, the pre-placodal region (PPR) that surrounds the neural plate. Within the PPR, otic and epibranchial precursors are located posteriorly, while lens and olfactory precursors are found more anteriorly. Surprisingly, we find that initially the entire PPR is specified as lens: even cells that normally never contribute to the lens begin to express lens-specific genes, when cultured in isolation, and form lens-like structures. This raises the possibility that lens represents a 'ground state' for all sensory placodes and that suppression of lens fate is required for other, neurogenic placodes to develop.